The *In Vitro* and *In Vivo* Effects of Human Growth Hormone Administration on Tumor Growth of Rats Bearing a Transplantable Rat Pituitary Tumor (7315b)

ARJEN BINNERTS,* PIET UITTERLINDEN, LEO J. HOFLAND, PETER v. KOETSVELD and STEVEN W.J. LAMBERTS

Department of Medicine and Clinical Endocrinology, Erasmus University, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands

Abstract—*The direct effects of human GH and IGF-1 on PRL secretion and cell proliferation were studied on PRL secreting rat pituitary tumor 7315b cells in vitro, as well as the effects in vivo of human GH administration on body weight, IGF-1 levels and tumor size in rats bearing this transplantable tumor. In the in vitro studies IGF-1 levels above 5 nM stimulated PRL release in a dose-dependent manner while GH, in concentrations of 0.23–45 nM, did not affect PRL release. Cell proliferation was stimulated by IGF-1 in a dose-dependent manner from 0.5 nM onwards, while GH did not have an effect. The in vivo studies showed that 1 mg GH/rat/day prevented tumor-induced cachexia and normalized the suppressed IGF-1 levels without stimulating tumor growth. It is concluded that tumor-induced cachexia can be prevented by exogenous GH administration without an increase in tumor mass, even if a tumor model is used whose cultured tumor cells respond to exposure to IGF-1 with a mitotic response.*

INTRODUCTION

IN ANIMALS, as well as in man, growth hormone (GH) exerts a wide variety of effects. Most of its growth-promoting actions are mediated via the formation of insulin-like growth factor I (IGF-I) while it also exerts a direct lipolytic action [1]. Knowledge about the effects and possible therapeutic uses of GH in adults has been limited by the lack of available supplies. Therefore during the past decades its use has mainly been restricted to the treatment of children with short stature.

After human GH, which is identical and bioequivalent to endogenous GH, was produced by recombinant DNA technology, new reports concerning the usefulness of GH therapy in adults with various catabolic disorders (trauma, surgery) were published [2–5]. To date no studies have been carried out on the potential beneficial anabolic effects of GH therapy in cancer patients.

Cancer-induced cachexia diminishes the quality of life significantly and may be attenuated by GH therapy. The amount of weight loss in cancer patients is positively correlated with the overall mortality. In addition, improvement of nutritional status may reduce chemotherapy related toxicity, as has recently been shown in a rat model [6]. The usefulness of (par)enteral (hyper)alimentation in the management of cancer patients is controversial [7–10].

Controversy exists also concerning the possible carcinogenic and/or tumor growth-stimulating effects of GH treatment in humans with GH deficiency, either direct or mediated by circulating or locally produced IGF-I [11–13]. According to some investigators the incidence of neoplasms in acromegaly is increased [14–17], though others did not observe this relation [18, 19].

In the present study we investigated the direct effects of human GH and IGF-I on prolactin (PRL)-secreting rat pituitary tumor 7315b cells *in vitro*, as well as the effects of human GH administration to rats bearing the transplantable rat pituitary tumor 7315b. In the *in vitro* experiments the effects on cellular DNA content and PRL secretion were investigated, while in the *in vivo* experiments changes in tumor size, body weight, plasma IGF-I, PRL and GH levels were studied.

*To whom all correspondence and reprint requests should be addressed.

MATERIALS AND METHODS

In vitro *tumor cell experiments*

Female Buffalo rats (R.B.I., Rijswijk, the Netherlands), weighing 150–170 g, were kept in an artificially illuminated room (08.30–20.30 h) with food and water *ad libitum*. The animals were inoculated subcutaneously between the scapulae with a cell suspension of the transplantable, PRL-secreting 7315b rat pituitary tumor as described in detail elsewhere [20]. Three to four weeks after inoculation of the tumor cell suspension a tumor of approximately 20 cm^2 has grown on the back of the animals. At this moment the animals were killed by an overdose of ether anesthesia and the tumor was carefully removed and collected in a sterile saline solution (9 g/l NaCl).

7315b pituitary tumor cells were isolated by mechanical dispersion. The isolated tumor was washed twice with calcium-, magnesium-free Hank's balanced salt solution (HBBS) supplemented with 1% human serum albumin (HSA), penicillin (10^5 U/l), streptomycin (100 µg/l), fungizone (0.5 mg/l) and sodium bicarbonate (0.4 g/l final concentration). The capsula of the tumor was carefully removed, after which the tumor was minced into small pieces. The remaining suspension of tumor tissue was gently vortexed for 30 s. After vortexing the suspension was centrifuged at 600 *g* for 5 min and the pellet was washed twice with HBSS + HSA. The remaining pellet was resuspended in HBSS + HSA and the suspension was filtered over a nylon gauze. In order to separate vital from non-vital cells the suspension was layered on Ficoll-Isopaque (density 1.077 g/ml; prepared by the Dijkzigt Hospital Pharmacy, Rotterdam, the Netherlands) and centrifuged at 500 *g* for 20 min. The interphase containing vital cells was collected and washed twice with HBSS + HSS. Finally, the cells were resuspended in culture medium.

The culture medium used in all experiments consisted of minimal essential medium with Earle's salts (MEM) supplemented with MEM non-essential amino acids, sodium pyruvate (1 mmol/l), 10% fetal calf serum, penicillin (10^5 U/l), streptomycin (100 µg/l), fungizone (0.5 mg/l), L-glutamine (2 mmol/l) and sodium bicarbonate (2.2 g/l final concentration). The medium was adjusted to pH 7.4 with 1 mol/l NaOH. The 7315b pituitary tumor cells were seeded at a density of 20,000 cells per well in 1 ml of culture medium in 24-well plates (Costar, Cambridge, Mass, U.S.A.) without or with IGF-I or GH. The number of replicate points is 4. After 6 days of culture the media and cells were collected and stored at −20°C until analysis. Medium and supplements were purchased from Grand Island Biological Co. Europe (Paisley, U.K.). IGF-I was obtained from Bachem (Bachem

Feinchemicalien ASG, Bubendorf, Switzerland) and human recombinant growth hormone (GH, Humatrope) from Eli Lilly & Co, Indianapolis.

Rat PRL concentrations in the culture media were measured by a double antibody RIA using materials and protocols supplied by the distribution officer of the NIADDK. All results are expressed in rat prolactin reference preparation-1 (RP-1).

The DNA content of the tumor cells was determined as described in detail elsewhere [21]. The method is based on a DNA-dependent fluorescence enhancement of a fluorochrome. In short, cultured tumor cells, which did not attach to the floor of the wells, were collected at the end of the incubation period and washed twice with an ice-cold saline solution. The remaining cell pellet was stored at −20°C until analysis. The cells were extracted with 300 µl ammonia solution (1 mol/l) + Triton X100 (0.2% v/v) by sonification during 5 s at amplitude 15 (Soniprep 150; MSE). Thereafter 2 ml assay buffer (100 mmol/l NaCl, 10 mmol/l EDTA, 10 mmol/l Tris; pH 7.0) was added. The remaining solution was centrifuged at 2000 *g* during 5 min and 100 µl aliquots of the supernatant were mixed with 1.5 ml Hoechst dye H33258 (100 ng/ml). Fluorescence was measured after 15 min with the exication and emission wavelengths set at 350 nmol/l and 455 nmol/l respectively. Fluorescence of experimental samples was referenced to a standard curve of calf thymus DNA (type II, no D-3636; Sigma Chemical Company, St Louis, Mo, U.S.A.).

Each experiment was done three times, the results shown relate to a single experiment. All data are expressed as mean ± S.E.

In vivo *tumor experiments*

In two experiments the effect of GH administration on the growth of the 7315b tumor and on reversal (experiment 1) or prevention (experiment 2) of tumor-induced cachexia was evaluated. Tumor growth was evaluated by expressing tumor size in centimeters squared (maximum length × maximum width) which has been shown to be closely correlated with tumor weight [22]. In the first experiment the effects of administration of 1 mg GH/rat/day (Humatrope, Eli Lilly & Co, Indianapolis) subcutaneously in 0.25 ml diluent starting on day 13 after tumor implantation until the end of the experiment (day 23) were evaluated, while in the second experiment the effects of GH administration for 15 days starting on the day of inoculation were evaluated. In both experiments tumor-bearing controls received daily injections of diluent only while in the second experiment the effects of either GH or diluent was evaluated in non-tumor-bearing controls as well. Each group of animals consisted of six rats.

Plasma IGF-I levels were measured in EDTA plasma obtained from the tail vein during the exper-

iment or after decapitation at the end of the experiment. The commercial kit for the determination of IGF-I from the Nichol's Institute of Diagnostics (San Juan Capistrano, CA, U.S.A.) was used. The intra-assay cv was 7.2 and the inter-assay cv 12.8%. PRL was determined in the same samples by double antibody RIA as described above.

Statistical evaluation

Statistical analysis was done by analysis of variance, followed by Duncan's test for determining the differences between control and experimental groups. In the *in vivo* experiments changes in body weight were evaluated by Student's unpaired *t* test.

RESULTS

The effects of IGF-I and GH on PRL secretion and DNA content of cultured 7315b tumor cells

The 7315b tumor cells were cultured for a period of 6 days. The effects of a wide concentration range of IGF-I (0.5–50 nmol/l) and GH (0.23–45 nmol/l) were investigated (Fig. 1). Low concentrations of 0.5 and 1 nmol/l IGF-I did not affect PRL release, while 5, 10 and 50 nmol/l IGF-I stimulated PRL release in a dose-dependent manner (5 nmol/l vs. control: $P < 0.01$; 10 vs. 5 nmol/l: $P < 0.05$; 50 vs. 10 nmol/l: $P < 0.05$). A low concentration of IGF-I (0.5 nmol/l) stimulated the DNA content of the cells after 6 days by 12% ($P < 0.05$ vs. control), while higher concentrations of IGF-I stimulated the

DNA content in a dose-dependent manner, 10 nmol/l being the maximal stimulatory concentration (stimulation by 1, 5, 10 and 50 nmol/l IGF-I being 24, 37, 50 and 55% respectively, 1 and 5 vs. 0.5 nmol/l: $P < 0.05$; 10 and 50 vs. 1 nmol/l: $P < 0.01$). We also measured the IGF-I concentration of the culture medium used in these experiments: the final IGF-I concentration of the medium to which the control cells were exposed amounted to 0.2 nmol/l.

Human GH in a concentration between 0.23 and 45 nmol/l did not affect PRL release, while it did also not influence the DNA content of the tumor cells after 6 days of culture (Fig. 1).

The effect of the administration of human GH in vivo on 7315b tumor growth, body and organ weights, serum PRL and IGF-I levels in rats

The daily, subcutaneous administration of a pharmacological dose of 1 mg GH/rat per day for 10–15 days was investigated on 7315b tumor growth in two separate experiments.

In the first experiment GH was injected daily from day 13 till 23 after tumor implantation. GH administration did not affect pituitary tumor growth: tumor size (as expressed in cm^2) did not differ from that found in tumor-bearing animals which received the diluent only (Fig. 2). In contrast, however, the GH-treated tumor bearing rats had gained weight considerably. In Fig. 2 it is shown that tumor-bearing control animals gained
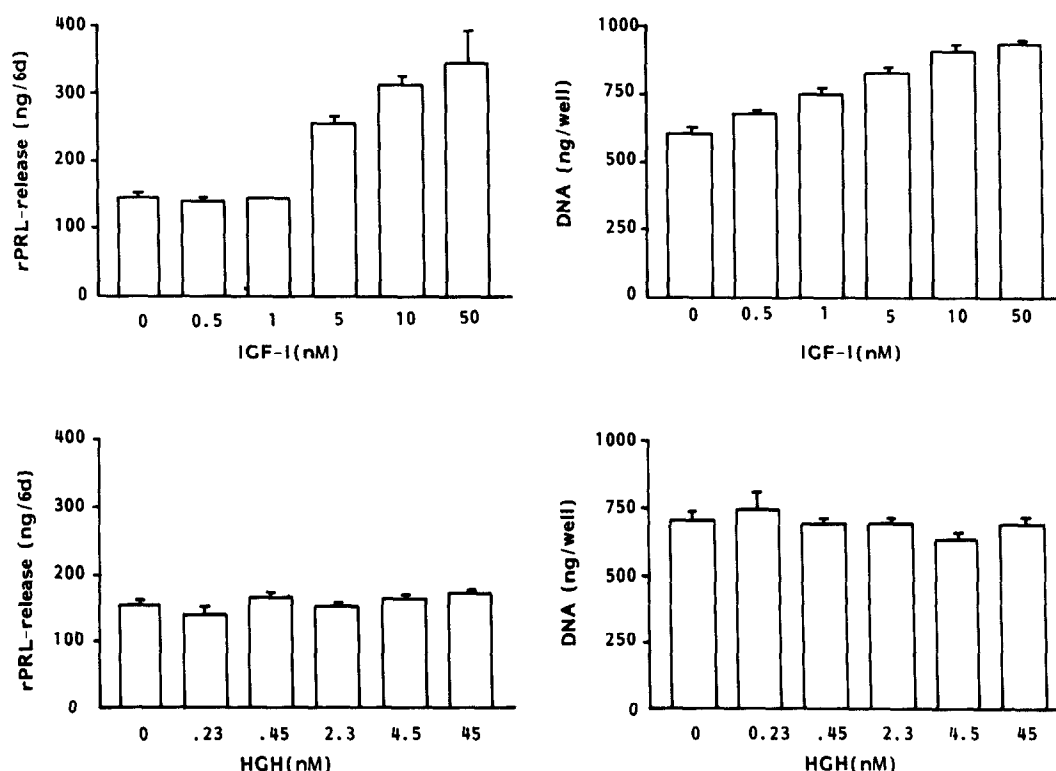

Fig. 1. *The effects of increasing concentrations of IGF-I (upper panels) and GH (lower panels) on rat PRL release (ng/6 days) and DNA content (ng/well) of 7315b tumor cells cultured for 6 days.*
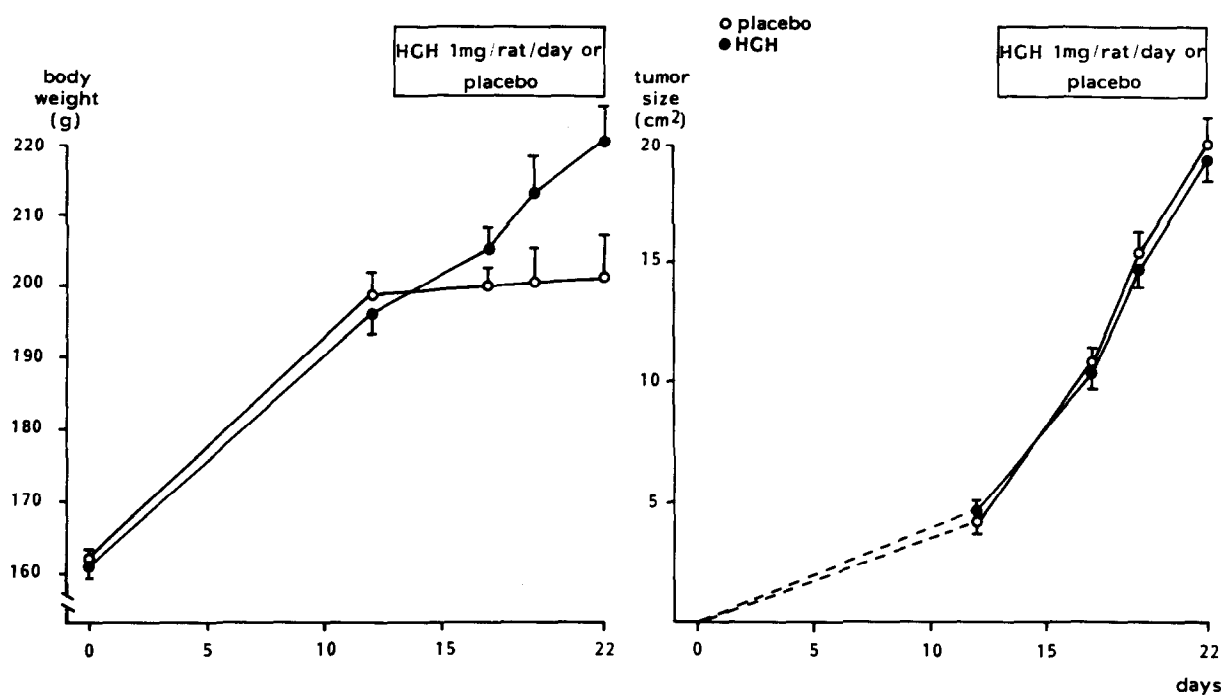
Fig. 2. The effects on body weight, uncorrected for tumor weight (left panel; g) and tumor size (right panel; cm²) of the administration of human GH (1 mg/rat/day) or placebo for 10 days to rats bearing the transplantable rat pituitary tumor 7315b starting on day 13 after tumor implantation. n = 6 for each group; mean ± S.E.M.

4.5 ± 6 g (mean ± S.E.M.) in weight during the 10 day placebo treatment period, while the GH treated tumor-bearing rats gained 24.3 ± 1 g.

After deduction of the mean tumor weight (35.1 ± 5 g in the placebo treated and 40.5 ± 2 g in the GH-treated animals; NS) the weight of the placebo-treated animals amounted to 166 ± 6 g, indicating a mean body weight gain of only 3 ± 2 g, in comparison with the mean body weight prior to tumor implantation. The actual mean body weight of the GH-treated group at the end of the experiment amounted to 180 ± 4 g (220.5 ± 5.4 minus 40.5 ± 2). Therefore the GH-treated animals had gained 18 ± 2 g in weight during the 22 day investigational period. This weight gain did not differ from that observed in non-tumor-bearing controls (starting weight 161 ± 2.8, final weight 175.3 ± 3.7) but was higher than that seen in the tumor-bearing control rats ($P < 0.01$). In comparison with non-tumor-bearing controls plasma IGF-I levels were slightly lowered on day 12 after tumor implantation (NS, Table 1a). This decrease was statistically significant in the tumor-bearing controls 18 and 22 days after tumor implantation ($P < 0.05$), while GH treatment in tumor-bearing animals resulted in a significant stimulation of IGF-I to levels which were comparable with those observed in non-tumor-bearing controls (both on day 18 and 22: $P < 0.05$ vs. tumor-bearing controls).

Prolactin levels in the GH treated tumor-bearing group did not differ significantly from the placebo-

treated tumor-bearing animals (1133 ± 156 vs. 771 ± 163 ng/ml), though both were significantly higher compared with the non-tumor-bearing controls (81 ± 32).

In the second experiment GH administration was started on the day of tumor implantation and continued for 15 days. Again no significant effect of GH administration on tumor growth was observed (Fig. 3). GH exerted powerful stimulatory effects on body growth both in the non-tumor- and in the tumor-bearing animals. The placebo-treated non-tumor-bearing rats gained 14 ± 2 g in weight, while in the GH-treated control group this amounted to 33 ± 3 g ($P < 0.01$). The tumor-bearing placebo-treated rats gained 34 ± 4 g in weight, but after deduction of tumor weight (26 ± 4 g) net weight gain was only 8 ± 2 g. Growth hormone administration to tumor-bearing animals resulted in a total weight gain of 49 ± 4 g; after deduction of tumor weight (29 ± 7): 20 ± 2 g ($P < 0.01$ vs. tumor-bearing placebo-treated rats).

After 15 days of GH treatment plasma IGF-I levels in the GH treated non-tumor-bearing rats were significantly elevated compared with the placebo-treated non-tumor-bearing animals ($P < 0.05$; Table 1b). The consequences of tumor implantation on IGF-I levels again became evident: both on day 16 and 22 they were significantly suppressed in comparison with the placebo-treated non-tumor-bearing rats ($P < 0.05$ in both instances). Administration of GH to tumor-bearing rats resulted already on day 9 but also on day 16 in a stimulation of

Table 1a. The effect of GH (1 mg/rat/day) or placebo administration on the total plasma IGF-1 concentration (nmol/l) of rats with the pituitary tumor 7315b, for 10 days starting on day 12 after tumor implantation (mean ± S.E.M.; n = 6 per group)

| | | IGF-I (nmol/l) | |
	Day 12	18	22
Control non-tumor	25.3 ± 1.6	23.6 ± 1.0	27.2 ± 2.4
Control tumor	23.1 ± 1.7	17.2 ± 1.1*	19.7 ± 0.9*
Tumor + GH	23.2 ± 0.8	27.7 ± 1.7†	28.5 ± 0.7†

*$P < 0.05$ vs. control non-tumor.
†$P < 0.05$ vs. control tumor.

Table 1b. The effect of GH (1 mg/rat/day) or placebo administration on the total plasma IGF-I concentration (nmol/l) of rats with the pituitary tumor 7315b, for 15 days starting on the day of tumor implantation (mean ± S.E.M.; n = 6 per group)

| | | IGF-I (nmol/l) | |
	Day 9	16	22
Non-tumor placebo	20.6 ± 1.0	22.2 ± 0.5	21.4 ± 1.0
Non-tumor + GH	23.9 ± 1.1	28.1 ± 0.6*	25.0 ± 1.0*
Tumor placebo	18.3 ± 0.7	16.9 ± 0.5*	14.1 ± 1.1*
Tumor + GH	23.8 ± 1.1†	25.9 ± 0.7†	15.8 ± 1.1

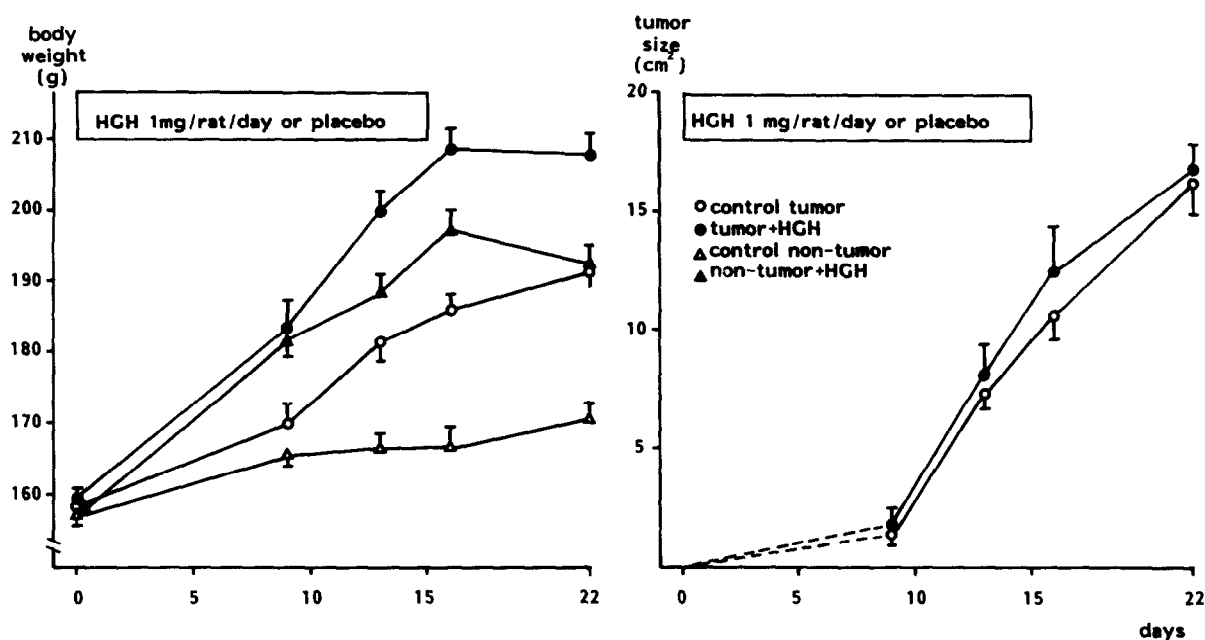*$P < 0.05$ vs. non-tumor placebo.
†$P < 0.05$ vs. tumor placebo.

Fig. 3. The effects on body weight, uncorrected for tumor weight (left panel; g) and tumor size (right panel; cm²) of the administration of human GH (1 mg/rat/day) or placebo for 15 days to rats with or without the transplantable rat pituitary tumor 7315b implanted on day 1. n = 6 for each group; mean ± S.E.M. On day 22 body weights corrected for tumor weight were 166 ± 5 in the control tumor and 178 ± 5 in the HGH treated tumor group.

IGF-I levels, which were significantly higher than in the placebo-treated tumor-bearing animals (P < 0.05). These levels were, both on day 9 and 16, similar to those observed in GH treated non-tumor-bearing rats, and on day 16 also higher than in placebo treated non-tumor-bearing controls (P < 0.05). On day 22 the stimulatory effects of GH administration in the first 15 day period had ceased and IGF-I levels had indeed decreased to levels comparable with those in the placebo-treated

tumor-bearing group. PRL levels in the GH-treated tumor-bearing group again did not differ significantly from the placebo-treated tumor-bearing rats (618 ± 195 vs 520 ± 103 ng/ml). PRL levels were elevated in both tumor-bearing groups compared with both non-tumor-bearing groups (PRL level 35 ± 6 in the non-tumor placebo-treated and 26 ± 7 ng/ml in the non-tumor GH-treated rats).

DISCUSSION

Evidence is accumulating that IGF-I plays a role in tumorigenesis and tumor growth. *In vitro* investigation of several tumor cell lines demonstrate specific binding sites for IGF-I [23–31], increased binding to IGF-I receptors when compared with less dedifferentiated cell lines or with normal surrounding tissue [24, 29–31], production of IGF-I by tumor cell lines or tissue [32, 33] and increased DNA synthesis and cell growth in response to IGF-I [23, 25, 27–29, 32, 34–36].

From these observations one might derive strong arguments against the therapeutic use of GH to reduce catabolism and/or induce an anabolic state in patients with cancer-induced cachexia, since it might simultaneously stimulate growth of the cancer itself. In addition evidence was also presented from studies by Friesen's group that GH directly induces the expression of the c-myc oncogene in rat liver [37].

Preliminary results (J. Foekens, personal communication) from investigations into the presence of IGF-I binding sites on the rat pituitary tumor cell line 7315b used in this study indicate indeed the presence of specific high affinity IGF-I binding sites on this tumor.

The results of the *in vitro* studies are in accordance with similar studies in other cancer cell lines, in that it was shown that IGF-I induced cell proliferation and protein (in this case PRL) secretion. GH itself did not induce either effect.

The results of the *in vivo* studies show that GH administration for 10–15 days prevents tumor-induced cachexia without stimulating tumor growth measured in cm^2 or PRL secretion. On a weight basis we used 120–240 times the GH dosage used

in human studies to induce an anabolic state [38].

The discrepancy between the (mitogenic) effect of IGF-I *in vitro* and the absence of effects of elevated IGF-I levels on tumor growth *in vivo* might be explained by the possibility with the low IGF-I levels in cachectic animals are sufficient to optimize tumor growth, since the lowest IGF-I level measured *in vivo* is 14 nM, while levels of 10 and 50 nM in the *in vitro* experiments stimulated cell proliferation to a similar extent. An auto- or paracrine effect of IGF-I generated by the tumor cells themselves cannot be excluded but seems unlikely since we found virtually no IGF-I production by these cells *in vitro* IGF-I (concentration in the control culture medium after 6 days 0.2 nM).

Our results are in disagreement with the results of Svaninger *et al.* [39], who demonstrated no improvement of body composition and muscle wasting (nor an increase in tumor growth) by GH administration in adult, non-growing, sarcoma-bearing mice, while in hypophysectomized rats GH administration stimulated body and tumor growth to a similar extent. In their sarcoma tumor model in intact animals GH levels were elevated from day 8 after tumor implantation, explained by the authors as a way to promote endogeneous substrate mobilization. The exogeneous GH dosage was only 100 μg/100 g body weight day (as opposed to our GH dose of 1 mg/rat, which corresponds to 600 μg/ 100 g body weight/day) which was insufficient to stimulate growth in freely fed control mice. In their study no IGF-I levels were reported.

In our study the decrease of IGF-I levels in tumor-bearing rats reflects the tumor induced cachexia [40. 41] and was reversible by GH administration. IGF-I levels decreased to the low level observed in untreated tumor-bearing rats within days after cessation of GH administration.

In conclusion, tumor-induced cachexia in this rat model can be prevented by exogenous GH administration without an increase in tumor mass, even though this tumor model contains specific binding sites for IGF-I and the cultured tumor cells respond to exposure to IGF-I with a mitotic response.

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